Genetic Polymorphisms of CYP2E1, GSTT1, GSTP1, GSTM1, ALDH2, and ODC and the Risk of Advanced Precancerous Gastric Lesions in a Chinese Population

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Abstract

There have been few studies of the associations of genetic polymorphisms with precancerous gastric lesions. We conducted a cross-sectional study to compare the prevalences of several genetic polymorphisms in 302 subjects with mild chronic atrophic gastritis with prevalences in 606 subjects with deep intestinal metaplasia or dysplasia. This stratified random sample of 908 subjects was selected and analyzed for genetic polymorphisms from 2,628 individuals who had gastric biopsies with histopathology in 1989 in Linqu County, Shandong Province, China. In subjects with mild chronic atrophic gastritis, the frequencies of the variant (less common) alleles of CYP2E1 *Rsa*I, CYP2E1 *Dra*I, GSTP1, ALDH2, and ODC were, respectively, 0.156, 0.201, 0.189, 0.190, and 0.428. The frequencies of the null genotypes of GSTM1 and GSTT1 in the mild chronic atrophic gastritis group were 0.509 and 0.565, respectively. Comparing mild chronic atrophic gastritis with deep intestinal metaplasia or any degree of dysplasia, we found no statistically significant associations with any genotype from these loci for dominant, additive, or recessive inheritance models. There was no statistically significant evidence of multiplicative interactions between any pair of genotypes based on CYP2E1 *Rsa*I, CYP2E1 *Dra*I, GSTP1, GSTM1, or GSTT1; nor between *Helicobacter pylori* status and any of these five loci; nor between smoking status and GSTP1, GSTM1, or GSTT1; nor between alcohol consumption and ALDH2. Statistically significant interactions were noted between salt consumption and GSTP1 and between sour pancake consumption and CYP2E1 *Rsa*I. There was, moreover, a statistically significant interaction (odds ratio, 1.78; 95% confidence interval, 1.03-3.08) between CYP2E1 *Dra*I and smoking at least one cigarette per day. A positive but not statistically significant interaction was also seen between CYP2E1 *Rsa*I and smoking status. These polymorphisms do not seem to govern progression from mild chronic atrophic gastritis to advanced precancerous gastric lesions, but the effects of smoking may be accentuated in individuals carrying variants of CYP2E1. (Cancer Epidemiol Biomarkers Prev 2005;14(2):451–8)

Introduction

Gastric cancer is one of the most common cancers in the world (1) and is the most common cancer in China (2). Linqu County, Shandong Province, China, has one of the highest mortality rates of gastric cancer in the world (rates age-adjusted to world standard of $70/10^5$ for males and $26/10^5$ for females, accounting for 42% of cancer deaths). A case-control study of gastric cancer in Linqu (3) and a subsequent survey with gastroscopies and biopsies of 3,400 subjects ages 35 to 64 years from 14 villages in Linqu County (4) identified several risk factors associated with gastric cancer and with precancerous gastric lesions, namely, in order of increasing severity, chronic atrophic gastritis, intestinal metaplasia, and dysplasia. In this region, chronic atrophic gastritis was nearly universal, intestinal metaplasia affected nearly half the subjects, and dysplasia affected 20% of adults (4). These risk factors motivated the selection of candidate genetic polymorphisms for this study of precancerous gastric lesions. Although several studies have evaluated polymorphisms in gastric cancer, little has been published on such associations with precancerous gastric lesions.

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The case-control study of gastric cancer in Linqu County (3) revealed increased risks from smoking cigarettes, consumption of sour pancakes that contain *N*-nitroso compounds, and consumption of salty foods. Family history of gastric cancer was also a risk factor. Consumption of fresh fruits and vegetables and allium vegetables (scallions, garlic, Chinese chives; ref. 5), which contain antioxidants, were protective. Calculations of micronutrient intake indicated protective associations with ascorbic acid, carotene, and calcium. Parallel studies of the prevalence of advanced precancerous gastric lesions (intestinal metaplasia or dysplasia) confirmed the positive associations with smoking and parental history of gastric cancer and also indicated some risk from consumption of alcohol and from having blood type A (6). Adverse associations were seen with consumption of sour pancakes, and protective associations with scallions and potatoes. Elevated serum levels of ascorbic acid and β-carotene were protective against intestinal metaplasia (7). Elevated gastric nitrite levels were associated with increased prevalence of intestinal metaplasia (8), and serum ascorbic acid was negatively correlated with a range of *N*-nitroso compounds in urine. Follow-up study of this population revealed that the risk of gastric cancer increases steadily with the severity of the baseline precancerous lesions (9) and that the odds of progression to dysplasia or gastric cancer was accelerated among smokers and among subjects with antibodies to *Helicobacter pylori* (10). Those in the highest tercile of serum ascorbic acid had a much reduced risk of progression to dysplasia/gastric cancer, with an odds ratio (OR) of 0.2 [95% confidence interval (CI), 0.1-0.7]. Thus, previous studies in this

high-incidence population identified several risk factors and protective factors that might be modulated by the specific polymorphisms selected for this study.

We selected candidate genes that had a plausible carcinogenic role either directly or by modifying the risks from exposures identified in earlier studies, had a functional variant with an effect on gene action, and had supportive population data, criteria outlined in ref. 11. CYP2E1 activates nitrosamines (12) that have been implicated in gastric carcinogenesis by some (13-15) but not all studies in Asian populations (16-18). Tobacco is a risk factor for gastric cancer, and both CYP2E1 and the glutathione *S*-transferase (GST) family of enzymes metabolize tobacco carcinogens and have been associated with tobacco-related cancers (19). Fruits and vegetables, including allium vegetables such as garlic and onions, have antioxidant activity that is influenced by GST enzymes (20), including GSTM1. The GST family is involved in the elimination of various electrophilic carcinogens (21), the genes are expressed in the gastrointestinal tract (22), and polymorphic variants of GSTM1, GSTT1, and GSTP1 have been investigated in relation to various cancers (23), with suggestive but still inconclusive results in gastric cancer (24). Alcohol has also been related to gastric cancer, and may induce CYP2E1 (25), whereas the carcinogenic metabolite acetaldehyde is detoxified by ALDH2. An inactive ALDH2 variant that is rare in Westerners but common in Asians raises the levels of acetaldehyde and increases the risk of upper aerodigestive cancers associated with drinking alcohol (26, 27). Elevated acetaldehyde levels can induce a flushing reaction, however, and may decrease the risk of excessive alcohol consumption (28). In addition, increased expression of ornithine decarboxylase (ODC) has been associated with gastric atrophy (29), and an ODC polymorphic variant has been recently associated with colon adenoma recurrence (30).

We therefore compared the prevalences of genotypes of CYP2E1, GSTP1, GSTM1, GSTT1, ALDH2, and ODC in a cross-sectional sample of subjects with deep intestinal metaplasia or dysplasia versus subjects with mild chronic atrophic gastritis. Except for the CYP2E1 *Dra*I variation (7632T>A) in the sixth intron (31), the genetic polymorphisms selected have functional significance. The CYP2E1 *Rsa*I polymorphic site (1053C>T) in the 5′-flanking region is associated with increased gene transcription *in vitro* (31). The ODC *Pst*I polymorphic site is in the promoter region and affects c-*myc* dependent ODC promoter activity (32). The ''null'' polymorphisms of GSTM1 and GSTT1 are large deletions, and homozygous carriers lack GST-μ and GST-θ activities, respectively (15, 33, 34). The missense polymorphism GSTP1 *Bsm*BI (313A>G) causes an isoleucine-to-valine change in the substrate binding region (35), and the ALDH2 *Mbo*II missense polymorphism at codon 487 changes glutamic acid to lysine, resulting in an inactive subunit (36).

Material and Methods

Study Population. From November 1989 through March 1990, a total of 3,433 subjects participated in a gastric cancer screening study, representing 83% of eligible residents ages 35 to 64 in 14 villages selected at random in Linqu County (6, 9). The study was approved by the Institutional Review Board of the National Cancer Institute and the Beijing Institute for Cancer Research, and all subjects gave a written informed consent. Three experienced gastroenterologists did the endoscopic examinations in 1989-1990, using fiber-optic gastroscopes (Olympus, Tokyo, Japan). The gastric mucosa was examined and seven biopsies were obtained from standard locations of the stomach, two in the body, one in the angulus, and four in the antrum. In the autumn of 1994, a repeat endoscopic examination using the same procedures as in 1989-1990 was offered to all cohort members (9).

Gastric Histopathology and Selection of Subjects. The same three Beijing Institute for Cancer Research pathologists made histopathologic diagnoses in 1989-1990 and 1994. The presence or absence of superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, or gastric cancer was recorded for each biopsy, and each subject was assigned a global diagnosis in 1989-1990 based on the most severe diagnosis among any of the seven biopsies; a global diagnosis was similarly defined for the seven biopsies obtained in 1994. Details of the pathologic procedures and classification criteria, along with photographs of superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, gastric cancer, and quality control procedures are provided elsewhere (6, 9). A total of 2,628 subjects had gastric histopathologic diagnoses both in 1989-l990 and 1994 (9).

The denominators in Table 1 give the numbers of subjects in categories defined by histology in 1989-1990 and in 1994. The numerators give the numbers of subjects who were randomly sampled within each category and for whom results from polymorphism assays were available. We assayed 302 subjects with mild chronic atrophic gastritis in 1989-1990 for comparison with subjects who had deep intestinal metaplasia (n = 307), or dysplasia (n = 299) in 1989-1990. Patients with severe chronic atrophic gastritis or superficial intestinal metaplasia were not sampled. Note that the sampling fractions were higher for categories with deep intestinal metaplasia or dysplasia in 1989-1990, for categories with fewer than 10 counts, and for categories with moderate or severe dysplasia or gastric cancer in 1994. This sampling scheme was used to obtain more precise information on longitudinal transitions among histologic states, while also allowing cross-sectional analyses. Reweighting of logistic analyses was done to eliminate bias from differential sampling fractions, and

Table 1. Distribution of subjects (denominator) and assayed samples (numerator) by histopathology in 1989-1990 and 1994

	1994 Histology							
1989-1990 Histology	Mild CAG	Severe CAG	Superficial IM	Deep IM	Mild DYS	Moderate or severe DYS	GC	Total sampled
Mild CAG	199/692	14/39	24/79	43/160	17/57	4/4	1/1	302/1032
Severe CAG	0/103	0/19	0/22	0/47	0/14	0/3	0/0	0/208
Superficial IM	0/83	0/12	0/32	0/97	0/30	0/0	0/2	0/256
Deep IM	48/93	8/9	31/60	140/279	62/119	7/10	11/16	307/586
Mild DYS	31/65	6/7	20/38	117/236	80/137	6/8	6/12	266/503
Moderate or severe DYS	5/6	0/0	3/4	14/17	9/11	1/2	1/3	33/43
								908/2628

NOTE: Although the analyses in the article refer to associations of polymorphisms with histopathology in 1989-1990, the subsample of the original 1989-1990 cross-sectional survey that was used to study polymorphisms was overweighted for those with severe histology in 1994. Corrections for overweighting are described in Materials and Methods.
Abbreviations: CAG, chronic atrophic gastritis; IM, intestinal metaplasia; DYS, dysplasia; GC, gastric cancer.

Table 2. Proportions (and SEs) of minor alleles and other factors by histopathology in 1989-1990

Factor	Mild CAG	Deep IM or DYS (APGL)	Two-sided *P*-value
Female	0.480 (0.040)	0.418 (0.026)	0.194
Ages ≥46 years	0.387 (0.039)	0.522 (0.023)	0.003
H. pylori positive	0.703 (0.034)	0.798 (0.028)	0.031
Smokes 20 or more cigarettes daily	0.287 (0.036)	0.350 (0.029)	0.174
GSTP1	0.189 (0.023)	0.197 (0.017)	0.806
CYP2E1 *Dra*I	0.201 (0.022)	0.244 (0.019)	0.131
CYP2EI RsaI	0.156 (0.019)	0.224 (0.017)	0.008
ALDH2	0.190 (0.023)	0.153 (0.016)	0.187
ODC	0.428 (0.029)	0.412 (0.022)	0.660

estimates of the proportions with various genotypes were also reweighted to account for varying sampling rates (Table 2). We obtained reweighted estimates of proportions separately for mild chronic atrophic gastritis, deep intestinal metaplasia, mild dysplasia, and moderate or severe dysplasia, corresponding to different rows in Table 1. The estimate for the combined category of deep intestinal metaplasia, mild dysplasia, and moderate or severe dysplasia was obtained as a weighted average of the three separate estimates, with weights 586/(586 + 503 + 43), 503/(586 + 503 + 43), and 43/(586 + 503 + 43). Variance estimates for proportions were obtained from the bootstrap sampling procedure described in the statistical methods.

Questionnaire Data. During the baseline examination in 1989-1990, each subject provided questionnaire information on tobacco and alcohol use, diet, socioeconomic status, and other variables (5).

***H. pylori* Antibody Assays.** *H. pylori* strains cultured from gastric biopsies of two patients in the study population in Linqu County were used to provide a local antigen preparation for serology. Serum *H. pylori* IgG and IgA antibody concentrations were each measured twice at baseline at Beijing Institute for Cancer Research in 1991-1992 with ELISA (37). Quality-control samples were assayed at Vanderbilt University. Each assay value was based on the mean of duplicate readings, and the mean of the two assay values was used to assess positivity. An individual was considered positive if the mean ELISA absorbance reading for either the IgG or IgA reading was above 1.0, a cutoff value based on *H. pylori*–negative persons and reference sera.

Blood Sample Collection. Blood samples were collected in the fall of 1997. The blood specimen was allowed to clot from 30 to 40 minutes at room temperature in a dark place. The clot was frozen immediately at −20°C and stored in a −70°C freezer within 2 or 3 days after collection. The clot samples were transferred on dry ice.

DNA Preparation. A total of 911 blood clots from 2,628 subjects were used to prepare genomic DNA, resulting in 908 successful DNA extractions. The blood clot was washed extensively with TE buffer (50 mmol/L Tris-HCl, pH 8.5, 1 mmol/L EDTA). After centrifugation, the pellet was incubated with rotation in a lysis buffer (TE buffer containing 0.2% SDS and 200 μg/mL proteinase K) for 2 hours at 55°C. The lysate was then extracted with phenol and precipitated with isopropyl alcohol. The precipitate was washed with 70% ethanol and dissolved in TE buffer. The purity and concentration of DNA were determined by spectrophotometry at A_{260nm} and A_{280nm}. DNA was aliquoted and stored in −80°C freezers with temperature alarms.

PCR Amplification and Determination of the Polymorphic Alleles. AmpliTaq Gold DNA polymerase was purchased from Roche Molecular Systems (Branchburg, NJ), and deoxynucleotide triphosphates were purchased from Amersham Pharmacia (Piscataway, NJ). NuSieve and SeaKem GTG agaroses were purchased from FMC (Rockland, ME). Restriction enzymes were purchased from New England BioLabs (Beverly, MA). Precast 20% polyacrylamide gel was purchased from Invitrogen (Carlsbad, CA).

PCR amplification was done on a GeneAmp PCR System 9700 and 2400 from Perkin-Elmer (Norwalk, CT). The reaction was started at 95°C for 5 to 10 minutes followed by 30 to 40 PCR cycles. The temperatures for denaturation, annealing, and elongation in each cycle were dependent on the polymorphic sequence variations. At the end, the reaction was extended to 7 minutes at 72°C.

Except for GSTM1 and GSTT1, the polymorphic alleles were determined by RFLP analysis, in which the PCR products were digested with restriction enzymes and analyzed by either agarose or PAGE. The presence of the common and variant alleles were designated as ''+'' and ''−'', respectively. The genotypes were then designated as ''+/+'' for homozygous common alleles, ''+/−'' for heterozygous variant alleles, and ''−/−'' for homozygous variant alleles.

CYP2E1 RsaI and DraI Polymorphisms. Genotyping for CYP2E1 *Rsa*I and *Dra*I polymorphism was conducted according to the methods reported by Hayashi et al. (31) and Hirvonen et al. (38), respectively. For the *Dra*I genotyping, we designed a reverse primer for PCR amplification (5′-CCA GGA AGG TCT CGA ACT CC-3′) to replace the original reverse primer in the reported method because the sequence of the latter did not match perfectly with the target region.

GST Genetic Polymorphisms. A multiplex PCR assay was used for detecting the ''null'' alleles, which was caused by gene deletion, of GSTM1 and GSTT1. In addition to the GSTM1 and GSTT1 primers, the same reaction mixture contained a pair of PCR primers to amplify a fragment of the histone gene, which served as a positive control for the PCR reaction. The null genotype for GSTM1 and GSTT1 was designated as ''−/−''. This assay did not distinguish between the homozygous (+/+) and heterozygous (+/−) genotypes for the common alleles. The GSTP1 *Bsm*BI polymorphism was determined by the method of Harries et al. (35).

ODC and ALDH2 Polymorphisms. The detection of ODC *Pst*I polymorphism was based on the method reported by Guo et al. (32), and the detection of the ALDH2 *Mbo*II polymorphism was based on the report by Yokoyama et al. (36).

Quality Control Procedures. Rigorous quality control procedures were applied throughout the genotyping process., The coding and analysis of DNA samples were double-blinded. Two investigators checked sample codes and data entry into the electronic database. To avoid PCR contamination, reagents for PCR reaction were carefully aliquoted and each aliquot was used no more than three times. Pipetting was done inside a cell culture hood. For each assay, a negative control (no DNA template) was added to monitor PCR contamination. Pilot experiments were always conducted to optimize the restriction digestion

conditions. After genotyping each genetic polymorphism, approximately 10% to 15% of the samples in each genotype group were randomly selected for repeated assays to validate the results. The average concordance rate was 99.9% (range, 97-100%).

Statistical Methods. The aim of the analysis was to detect an association between various genotypes and the presence of advanced precancerous gastric lesions (APGL), namely, deep intestinal metaplasia or any form of dysplasia in 1989-1990 versus mild chronic atrophic gastritis in 1989-1990. The logistic statistical model used for analysis was

$$logit\ P(APGL) = \mu + X(g)\beta + Z\gamma, \qquad (A)$$

where Z represents covariates such as age in 1989, gender, and number of cigarettes smoked per day in 1989. The quantity X(g) is a score associated with the genotypes g = +/+, +/−, or −/−. For a dominant model X(g) = 1 if g = −/− or +/− and 0 otherwise. For a recessive model, X(g) = 1 if g = −/− and 0 otherwise. For an additive model X(g) = 0 for g = +/+, 1 for g = +/−, and 2 for g = −/−. We tested each model for each genetic polymorphism using a two-sided test based on a standardized normal deviate as described below. To account for multiple testing, we also present a P value for the maximum of the three absolute values of the deviates. For some assays, such as GSTM1 and GSTT1, one can only distinguish g = −/− from g = +/+ or +/−; hence, only the recessive model could be tested. The logistic model above, but with product terms, was used to test for interactions between an additive coding of a genotype from one locus with an additive coding of the genotype from another locus or with a main effect of a covariate such as smoking. For GSTM1 and GSTT1, recessive codings were used instead of additive codings.

To account for the unequal sampling fractions in Table 1, we did a weighted analysis, using SAS PROC LOGISTIC (39). For example, the 199 observations in the upper left cell of Table 1 were given a sampling weight proportional to 692/199, whereas the 1 observation in the lower right cell was given a weight proportional to 3/1. Those with severe chronic atrophic gastritis and superficial intestinal metaplasia were ignored in these analyses.

To obtain P values, variance estimates, and CIs, we used the following bootstrap procedure. For each bootstrap sample, we resampled the denominators in Table 1 with replacement to obtain 2628 − 208 − 256 = 2164 new counts distributed as a multinomial in the table. More precisely, we resampled the observations in row 1 with replacement to obtain 1032 ''new'' observations, and we did the same for rows 4, 5, and 6. Holding the sampling fractions from the original Table 1 constant, we calculated the number to be sampled for genotyping (numerators) in each cell of the new table. In each cell, we then resampled that new number of samples with replacement from the original sample of genotyped individuals. We then did all the statistical tests and recorded the results for that bootstrap repetition. We used B = 10,000 bootstrap repetitions and computed P values from the standard normal deviate $Z = \hat{\beta}/\hat{\sigma}$, where $\hat{\beta}$ is the original estimate of β, and $\hat{\sigma}$, the estimated standard error of $\hat{\beta}$, is obtained from $\hat{\sigma}^2 = 1/(B-1)\Sigma(\hat{\beta}_b - \bar{\beta})^2$. Here, $\hat{\beta}_b$ is the estimate of β in bootstrap repetition b, and $\bar{\beta} = B^{-1}\Sigma\hat{\beta}_b$. To compute the P value associated with the maximum absolute standardized deviate associated with the three tests, we obtained the covariance matrix of the three standardized deviates from the bootstrap samples. We then obtained 1,000,000 samples from a trivariate normal distribution with mean zero and this covariance to obtain the distribution of the maximum absolute standardized deviate. The P value was the proportion of this distribution that exceeded the maximum absolute standardized deviate in the original data. Two-sided 95% CIs were obtained from the quantiles of the bootstrap distribution of $\hat{\beta}_b$.

Table 3. Weighted proportions (with SEs) of genotypes for subjects with mild CAG and for subjects with deep IM or DYS in 1989-1990, and ORs and *P* values for testing for an association of histopathology with genotype

	Mild CAG	Deep IM or DYS (APGL)	OR* (95% CI)	$P^\dagger$			
				Dominant	Additive	Recessive	Max
CYP2E1 (*Rsa*I)							
+/+	0.711 (0.031)	0.584 (0.029)	1.21 (0.93, 1.57)	0.61	0.16	0.16	0.31
+/−	0.265 (0.029)	0.384 (0.030)					
−/−	0.024 (0.013)	0.032 (0.012)					
CYP2E1 (*Dra*I)							
+/+	0.634 (0.037)	0.563 (0.029)	1.12 (0.87, 1.44)	0.87	0.37	0.32	0.54
+/−	0.330 (0.036)	0.385 (0.030)					
−/−	0.036 (0.013)	0.052 (0.015)					
GSTP1							
+/+	0.667 (0.037)	0.631 (0.030)	0.93 (0.72, 1.21)	0.78	0.60	0.42	0.66
+/−	0.288 (0.035)	0.346 (0.029)					
−/−	0.045 (0.017)	0.024 (0.007)					
GSTM1							
+/+ or +/−	0.491 (0.039)	0.491 (0.029)					
−/− (null)	0.509 (0.039)	0.509 (0.029)	1.12 (0.85, 1.49)			0.42	
GSTT1							
+/+ or +/−	0.435 (0.038)	0.443 (0.027)					
−/− (null)	0.565 (0.038)	0.557 (0.027)	1.15 (0.87, 1.54)			0.33	
ALDH2							
+/+	0.687 (0.032)	0.713 (0.028)	0.93 (0.71, 1.21)	0.77	0.58	0.38	0.63
+/−	0.245 (0.032)	0.268 (0.027)					
−/−	0.067 (0.021)	0.019 (0.009)					
ODC							
+/+	0.295 (0.037)	0.365 (0.028)	0.93 (0.76, 1.14)	0.47	0.49	0.12	0.23
+/−	0.554 (0.037)	0.447 (0.029)					
−/−	0.151 (0.028)	0.189 (0.023)					

*The ORs (95% CIs) comparing deep intestinal metaplasia or dysplasia with mild chronic atrophic gastritis correspond to a unit increase in the number of variant alleles under the additive model. For GSTM1 and GSTT1, the OR compares the homozygous variant (null) genotype with other genotypes.
†All analyses are adjusted for age (continuous) and gender. ''Max'' indicates the P value associated with the maximum of the absolute values of the three standardized deviates corresponding to dominant, additive, and recessive models.

Results

Information on gender, age, and pathology at baseline in 1989-1990, and genetic polymorphisms was available on 908 subjects (Table 1). Information on these variables and on current smoking status (0, 1-19, or 20 or more cigarettes per day), *H. pylori* seropositivity, and alcohol consumption was available for 672 subjects. Information on consumption of salt and of sour pancakes was available on 532 and 541 subjects, respectively. Unreported weighted logistic analyses indicated that age, presence of antibodies to *H. pylori*, and cigarette smoking were significantly associated with APGLs. Positive but nonsignificant associations of APGLs were seen with male gender, sour pancake consumption, and consumption of more than one drink of hard liquor per week.

Weighted estimates of the variant (less common) allele frequencies are shown in Table 2, separately for those with APGLs and those with mild chronic atrophic gastritis, along with weighted estimates of the proportions with other characteristics. There were no significant differences between the APGL and mild chronic atrophic gastritis groups in the male-to-female ratio or in the frequencies of the variant alleles of GSTP1, CYP2E1 *Dra*I, ALDH2, or ODC (Table 2). The proportions of subjects older than 45 years of age and of those seropositive for *H. pylori* were significantly higher in the APGL group, and there was a nonsignificant tendency for smokers of 20 or more cigarettes daily to be more frequent in the APGL group. The proportion of variant (−) alleles of CYP2E1 *Rsa*I was higher in the APGL group than among the mild chronic atrophic gastritis group (Table 2).

The weighted estimates of frequencies of various genotypes are shown for the APGL and mild chronic atrophic gastritis groups (Table 3). The (−/−) genotypes of CYP2E1 *Rsa*I, CYP2E1 *Dra*I, and GSTP1 had frequencies under 5% in the mild chronic atrophic gastritis group, and the ALDH2 (−/−) genotype was also uncommon. From data in Table 3, we estimated the variant (less common) allele frequencies in the mild chronic atrophic gastritis group for CYP2E1 *Rsa*I, CYP2E1 *Dra*I, GSTP1, ALDH2, and ODC to be 0.156, 0.201, 0.189, 0.190, and 0.428, respectively. The frequencies of the null genotypes of GSTM1 and GSTT1 in the mild chronic atrophic gastritis group were 0.509 and 0.565, respectively.

No statistically significant associations with APGL were found with any of the genotypes studied, regardless of the heritability model used (Table 3). The associations were even less impressive when one uses the *P* value under Max in Table 3, taking into account examination of dominant, additive, and recessive models. ORs for a unit increase of variant alleles under the additive genetic model were near unity for CYP2E1 *Rsa*I, CYP2E1 *Dra*I, GSTP1, ALDH2, and ODC. Likewise, the ORs associated with having the null GSTM1 genotype and the null GSTT1 genotype were near unity. These analyses were adjusted for age (continuous) and gender, but similar results were obtained for the unadjusted data (not shown). Analyses fully adjusted for age, gender, *H. pylori* status, and smoking status likewise failed to show any association with these polymorphisms (data not shown).

Further analysis combined the possible effects of the various polymorphisms by assigning to each subject the following score: one point if GSTT1 is null; plus one point if GSTM1 is null; plus the number of minor alleles in the ALDH2, GSTP1, and ODC genotypes; plus half the number of minor alleles in the CYP2E1 *Rsa*I and CYP2E1 *Dra*I genotypes. This score thus ranged from 0 to 10. The OR relating a unit increase in this score to APGL was 0.99 (95% CI, 0.89-1.10) with $P = 0.81$, indicating no significant association between this combined risk score and APGL.

Because a variant of ALDH2 may cause some individuals to stop drinking alcohol because of flushing reactions (28), we looked for associations with the variant of ALDH2 in the subset of 394 subjects who drank hard liquor at least once a week. Unadjusted analyses and analyses adjusted for age and gender revealed no association with the ALDH2 variant under additive, dominant, or recessive models.

To evaluate possible interactions between pairs of the five genes CYP2E1 *Rsa*I, CYP2E1 *Dra*I, GSTP1, GSTM1, and GSTT1, we used additive scores for main effects of CYP2E1 *Rsa*I, CYP2E1 *Dra*I, and GSTP1 and recessive scores for GSTM1 and GSTT1, and we included an interaction between theses scores in the logistic model (1) for each of the 10 possible pairs of genes. All analyses were adjusted for age and gender. None of the interactions was large, and none reached statistical significance (Table 4).

Cigarette smoking showed no significant interaction with CYP2E1 *Rsa*I, GSTP1, GSTM1, GSTT1, or ALDH2 (Table 5). There was a modest (OR, 1.78) but significant interaction between CYP2E1 *Dra*I and smoking at least one cigarette per day (but not ≥20 cigarettes per day; Table 5), suggesting that the risks from smoking may be greater among those with variant CYP2E1 *Dra*I alleles. A similar tendency, but not statistically significant, was seen for CYP2E1 *Rsa*I and smoking at least one cigarette per day (Table 5). There was no evidence of interaction with *H. pylori* seropositivity nor between having at least one hard alcoholic beverage per week and ALDH2 or other polymorphisms in Table 5. There was a weak but statistically significant interaction (OR, 1.46) between salt consumption and GSTP1, but not with other polymorphisms (Table 5). A stronger statistically significant interaction (OR, 2.43) was found between sour pancake consumption and CYP2E1 *Rsa*I, but not with other polymorphisms (Table 5).

Among the 19 subjects with gastric cancer diagnosed in 1994, the distributions of genotypes were similar to those in Table 3 (data not shown), but the proportions of (−/−) genotypes of CYP2E1 *Rsa*I and CYP2E1 *Dra*I, 0.053 and 0.105, were larger than for the mild chronic atrophic gastritis subjects in Table 3. These differences were not statistically significant, however, and the numbers were too small to draw firm conclusions.

Discussion

Considerable information is available on the prevalence of somatic mutations in gastric cancer (40-42) and in precursor lesions (41-44), and on germ line polymorphisms in subjects with gastric cancer and controls (13, 14, 16-18, 23-24, 26, 33-34, 45-49). Little has been published, however, on associations between inherited polymorphisms and precancerous gastric

Table 4. Estimates of gene-gene interactions, adjusted for age and gender

	Estimated interaction OR (95% CI)	*P*
CYP2EI *Rsa*I		
xCYP2EI *Dra*I	1.09 (0.67, 1.76)	0.73
xGSTM1*	1.28 (0.75, 2.17)	0.37
xGSTT1*	1.14 (0.67, 1.96)	0.62
xGSTP1*	0.79 (0.49, 1.28)	0.34
CYP2E1 *Dra*I		
xGSTP1	0.79 (0.49, 1.26)	0.31
xGSTM1*	1.02 (0.61, 1.71)	0.93
xGSTT1*	1.14 (0.69, 1.88)	0.61
GSTP1		
xGSTM1*	0.75 (0.44, 1.30)	0.31
xGSTT1*	1.26 (0.74, 2.14)	0.39
GSTM1*		
xGSTT1	0.70 (0.39, 1.25)	0.23

NOTE: Genotypes with asterisks are coded as 1 for the null genotype and 0 otherwise. Genotypes without asterisks are coded as 0 for +/+, 1 for +/−, and 2 for −/−, where − indicates a variant (less common) allele and + a common allele.

Table 5. Interactions of genotypes with smoking, *H. pylori* seropositivity in 1989, salt consumption, sour pancake consumption, and drinking alcohol, adjusted for age and gender

	Estimated interaction OR (95% CI)	*P*
Smoking at least 1 cigarette daily		
xCYP2E1 *Rsa*I	1.46 (0.81, 2.61)	0.20
xCYP2EI *Dra*I	1.78 (1.03, 3.08)	0.04
xGSTP1	0.79 (0.46, 1.37)	0.40
xGSTM1*	1.01 (0.56, 1.79)	0.99
xGSTT1*	1.42 (0.79, 2.56)	0.24
xALDH2	1.15 (0.65, 2.02)	0.63
Smoking at least 20 cigarettes daily		
xCYP2E1 *Rsa*I	1.06 (0.56, 2.04)	0.85
xCYP2E1 *Dra*I	1.07 (0.60, 1.92)	0.81
xGSTP1	0.90 (0.50, 1.61)	0.73
xGSTM1*	1.04 (0.53, 2.06)	0.90
xGSTT1*	1.36 (0.71, 2.62)	0.36
xALDH2	1.09 (0.56, 2.09)	0.81
H. pylori seropositive in 1989		
xCYP2E1 *Rsa*I	1.02 (0.50, 2.09)	0.95
xCYP2EI *Dra*I	0.95 (0.50, 1.80)	0.88
xGSTP1	1.06 (0.56, 2.02)	0.85
xGSTM1*	0.96 (0.45, 2.04)	0.92
xGSTT1*	0.59 (0.29, 1.22)	0.15
xALDH2	0.73 (0.33, 1.59)	0.43
Drinking hard liquor at least once per week		
xCYP2E1 *Rsa*I	1.15 (0.66, 2.01)	0.62
xCYP2E1 *Dra*I	1.33 (0.79, 2.24)	0.29
xGSTP1	1.21 (0.68, 2.15)	0.51
xGSTM1*	1.30 (0.72, 2.37)	0.38
xGSTT1*	1.38 (0.75, 2.55)	0.31
xALDH2	0.71 (0.37, 1.36)	0.30
Salt use		
xCYP2E1 *Rsa*I	1.00 (0.70, 1.42)	0.99
xCYP2EI *Dra*I	0.91 (0.65, 1.28)	0.59
xGSTP1	1.46 (1.06, 2.03)	0.022
xGSTM1*	0.91 (0.64, 1.30)	0.60
xGSTT1*	0.90 (0.63, 1.30)	0.59
xALDH2	0.75 (0.53, 1.07)	0.11
Consumption of sour pancakes		
xCYP2E1 *Rsa*I	2.43 (1.10, 5.38)	0.029
xCYP2E1 *Dra*I	1.46 (0.70, 3.05)	0.31
xGSTP1	0.96 (0.44, 2.12)	0.92
xGSTM1*	0.75 (0.30, 1.85)	0.53
xGSTT1*	1.43 (0.59, 3.46)	0.43
xALDH2	0.66 (0.32, 1.37)	0.26

NOTE: Genotypes with asterisks are coded as 1 for the null genotype and 0 otherwise. Genotypes without asterisks are coded as 0 for +/+, 1 for +/− and 2 for −/−, where − indicates a variant (less common) allele and + a common allele. Salt use is coded as 0, 1, 2, or 3, respectively, for annual salt use of less than 4.5, 4.5-5.5, 6-8, and >8 kg per year. Sour pancake consumption is coded as 0, 1, or 2, respectively, for 0, 1-180, or >180 kg of pancakes per year.

lesions. Our relatively large sample was selected at random from a well-defined high-incidence population and allowed a cross-sectional evaluation of associations between genetic polymorphisms and APGL (deep intestinal metaplasia or dysplasia). This sample also enabled an assessment of combined effects of genetic polymorphisms and of gene-exposure interactions on the risk of APGL. In addition, questionnaire data on diet, smoking, and alcohol consumption were collected before histopathologic diagnoses were available, reducing the possibility of differential recall bias. We were also able to control for confounding factors such as age, gender, and cigarette consumption.

Analyses that controlled for gender and age revealed no statistically significant associations with APGL, whether or not adjusted for gender, age, smoking, and *H. pylori* seropositivity. Our study was sufficiently large to yield precise estimates of genetic effects, as indicated by the widths of the CIs in Table 3. In fact, none of the upper confidence limits exceeded 1.6. Thus, it is unlikely that our study failed to detect genetic ORs of 1.6 or more for the null genotypes of GSTM1 or GSTT1 or for an increment of one variant (minor) allele in an additive genetic model on the logistic scale for the other genotypes in Table 3. The powers of these studies to detect an OR of 1.5 under the additive model were 0.90, 0.94, 0.93, 0.93, and 0.98 for CYP2E1 *Rsa*I, CYP2E1 *Dra*I, GSTP1, ALDH2, and ODC, respectively, based on calculations in ref. 50. Thus, it is unlikely that this study failed to detect strong main effects of these polymorphisms.

Several other explanations, apart from chance, might account for failure to detect associations. Because there were very few subjects with normal gastric mucosa in this population, we used the mild chronic atrophic gastritis group for comparison with APGL. If these variants affected only transitions from normal mucosa to mild chronic atrophic gastritis or from APGL to gastric cancer, their effects would not be detected by this design. In addition, if the variants studied were not disease-producing alleles, but were in linkage disequilibrium with a putative disease allele, then the power of the study to detect such an association would be severely attenuated (50). Another limitation is the possible role of other carcinogenic factors and pathways that could reduce the power to detect the effects of any one variant (51). In addition, because there are few strong associations with environmental risk factors, it seems likely that polymorphisms that modulate effects of these exposures would have modest effects, perhaps too small to show even with a study of over 900 subjects.

The published data relating polymorphisms to gastric cancer are relevant to our findings. Wu et al. (33) found an association of gastric cancer with CYP2E1 *Rsa*I, but not with CYP2E1 *Dra*I. On the other hand, Park et al. (13) and Tsukino et al. (18) found no such association. Neither Deakin et al. (49), nor Katoh et al. (34) nor Wu et al. (33) found associations with the null genotype of GSTT1, but Setiawan et al. (24) reported such an association in two Chinese populations. The null genotype of GSTM1 was associated with gastric cancer in one study (49), with an OR of 1.70 (95% CI, 1.05-2.76), but not in another study (33). We could not find reports relating ODC polymorphisms to gastric cancer, although levels of ODC mRNA expression were elevated in gastric cancer tissue compared with adjacent normal tissue (52). Yokoyama et al. (53) reported higher frequencies of the variant allele of ALDH2 in 56 patients with gastric cancer than in 487 cancer-free Japanese alcoholics. Thus, the data from studies of gastric cancer are conflicting or offer only modest evidence for associations with the polymorphisms we studied. It is not surprising, therefore, that we failed to detect an association, even though our study was larger than many published studies on gastric cancer.

Even larger sample sizes are required to detect gene-gene or gene-environment interactions (54), and our study does not rule out appreciable interactions among genes (Table 4) or with environmental factors (Table 5) because CIs are wider than for main effects (Table 3). Three interactions were nominally significant (Table 5), including an interaction between CYP2E1 *Dra*I and smoking at least one cigarette daily (Table 5). Because several interactions were examined, this association needs to be confirmed in independent studies of precancerous gastric lesions. It is also true that the interaction was only seen when comparing smokers with nonsmokers and not when the smoking population was dichotomized at 5, 10, or 20 cigarettes per day (Table 5).[5] However, this finding is supported by studies of the percentage of (−/−) CYP2E1 *Dra*I genotypes in nonsmoking, light-smoking, and heavy-smoking patients with gastric cancer (13). We analyzed the data from Table 2 of Park et al. (13) and detected a linear-by-linear interaction (exact *P* value of 0.0035), indicating a positive association between the

[5] Unpublished analyses.

number of minor alleles (c2 alleles) and the amount of smoking. This ''case-only'' analysis supports our finding of an interaction between CYP2E1 *Dra*I and smoking, but is not as robust as our demonstration of an interaction using cases and controls because it relies on an assumption of independence between smoking and CYP2E1 *Dra*I in the source population (55).

We found an interaction between salt use and GSTP1 (Table 5). Chen et al. (15) present some evidence (although no formal tests for interaction) that risks of ''incomplete'' intestinal metaplasia from salted meat and seafood are enhanced in subjects with the GSTT1 null genotype, the GSTM1 nonnull genotype, and in subjects homozygous for CYP2E1 *Rsa*I minor alleles. Our data, in contrast, show no evidence of statistically significant interactions with GSTT1, GSTM1 or CYP2E1 *Rsa*I (Table 5). The study by Chen et al. included only subjects with medical indications for gastroscopy, and the findings were based on only 35 cases with incomplete intestinal metaplasia, resulting in wide CIs. Our analysis of salt use rather than consumption of specific salted foods, and the other differences in study design may account for the discrepancies.

We also noted an interaction between consumption of sour pancakes and CYP2E1 *Rsa*I minor alleles (Table 5). It is tempting to speculate that this interaction might be related to CYP2E1 activation of nitrosamine constituents or other carcinogenic components of sour pancakes. Assays of sour pancakes from Linqu for *N*-nitroso compounds and Ames tests were unimpressive, however (56), as were tests for fumonisins, which are mycotoxins (57).

In summary, this population-based study provided no evidence that variants of candidate gene polymorphisms (CYP2E1 *Rsa*I, CYP2E1 *Dra*I, GSTP1, GSTM1, GSTT1, ODC, ALDH2) that may modulate the risk factors identified in this high-incidence area of China are more common in subjects with intestinal metaplasia or dysplasia than in those with mild chronic atrophic gastritis. However, we did find an interaction between cigarette smoking and CYP2E1 *Dra*I that, together with data from gastric cancer cases (13), suggests that CYP2E1 *Dra*I interacts with smoking to increase the risk of advanced precursor lesions on the pathway to gastric cancer.

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